POPULATIONS OF FUNGI IN SOME RESERVOIRS IN SERBIA

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ABSTRACT : The paper presents results of mycological studies conducted in the three larger reservoirs in Serbia (Ćelije, Grošnica and Gruža), which have different hydrological and production characteristics. These studies were conducted during all seasons of 1996. The quantitative analysis of fungal communities showed that the average number of their spores in these reservoirs was between 2000 and 4990 per liter of water. The highest number of spores was found in eutrophic reservoir of Gruža and the lowest identified in oligo-mesotrophic reservoir of Ćelije. Number of spores was higher in the water samples taken near the bottom of the lakes and in littoral zone rich with macrovegetation, than in the middle water layers. In these reservoirs 45 species were identified out of 384 isolates. The most dominant genera were *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Phoma*, *Rhizopus* and *Verticillium*. The autochthonous aquatic fungal community was composed of the following species:*Achlya americana*, *Achlya racemosa*, *Catenaria sp.*, *Dictyuchus sterile*, *Pythium ultimum*, *Saprolegnia ferax*, *Saprolegnia hypogyna* and *Saprolegnia monica*.

KEY WORDS : allochthonous and autochthonous aquatic fungi, reservoir.

RÉSUMÉ : Le présent article résume les études mycologiques réalisées sur les trois plus impportants réservoirs de Serbie (Ćelije, Grošnica et Gruža), qui possèdent des caractéristiques hydrologiques différentes. Ces études ont été effectuées durant l'année 1996. Les analyses quantitatives des communautés fongiques indiquent que le nombre moyen de spores par litre d'eau est compris entre 2000 et 4990 ; la valeur la plus élevée correspondant au réservoir eutrophique de Gruža et la plus basse au réservoir oligo-mésotrophique de Ćelije. Le nombre de spores est plus important dans les échantillons prélevés à proximité du fond du lac et dans la frange littorale riche en macrovégétation, que dans les prélèvements effectués en moyenne profondeur. Dans ces réservoirs 45 espèces ont été identifiées sur un total de 384 isolats. Les genres dominants sont : *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Phoma*, *Rhizopus* et *Verticillium*. La communauté fongique aquatique autochtone était constituée des espèces suivantes : *Achlya americana*, *Achlya racemosa*, *Catenaria sp.*, *Dictyuchus sterile*, *Pythium ultimum*, *Saprolegnia ferax*, *Saprolegnia hypogyna* et *Saprolegnia monica*.

MOTS CLÉS : champignons aquatiques allochtones et autochtones, réservoirs.

INTRODUCTION

Aquatic fungi play important role in the transformation processes of organic matter (Park, 1972a) in biohydrocenoses. They affect the numbers of other hydrobionates (algae, protozoans, insects etc.) causing their epiphytocia and accumulation of dead

organic matter in ecosystem (Sen, 1988a, 1988b). They participate in destruction of autochthonous and allochthonous organic matter, providing food for some invertebrates and fish (Bärlocher, 1980, 1981) and also cause the water self-cleaning processes (Hynes *et al.*, 1974). The fungi have not been extensively studied in the aquatic ecosystems of Serbia (Ranković *et al.*, 1994; Vukojević & Franić-Mihajlović, 1994; Čomić *et al.*, 1995). The important role played by the aquatic fungi as structural and functional components of biohydrocenoses, as well as the fact that their research in Serbia is just beginning, have motivated us to conduct these studies.

The studies were conducted in the three larger reservoirs in Serbia (Ćelije, Grošnica and Gruža), which have different hydrological and production characteristics. These reservoirs belong to different classification types (Seleši, 1989; Martinović-Vitanović & Kalafatić, 1990; Rankovic *et al.*, 1994). Accordingly, reservoir Ćelije belongs to oligotrophic-mesotrophic type, reservoir Grošnica belongs to mesotrophic type and reservoir Gruža belongs to eutrophic type. The qualitative and quantitative composition of the allochthonous and autochthonous fungal communities, distribution of isolates obtained and seasonal dynamics were monitored in the period of study.

MATERIALS AND METHODS

During 1996, water samples were collected from the reservoirs during all seasons of the year (March-M, June-J, August-A, October-O) on 4-5 sites in the central parts of the reservoirs. The samples were taken from the layer near the bottom of the reservoirs (L1), middle layer (L2) in the central parts of the reservoirs and from the area near the shore which was full of plants at 20-30 cm depth (L3). Water samples were collected with a 2 liter Ruttner sampler. Water samples were processed during the same day of their collection using the dilution plate technique, adding 2 ml of water on Malt-agar culture medium in Petri dish with three repetitions. The quantity of fungal spores was determined by the number of the grown colonies. These were then isolated in pure cultures using standard mycological methods and replating on the selective culture media. All cultures were incubated at 25C ($\pm$ 2) under day-night light exposure. Stock cultures are kept in the culture collection of the Faculty of Science, University of Kragujevac.

The autochthonous, aquatic fungi were studied by direct microscopic examination of the water and material collected by a baiting method with seeds of *Canabis sativa*, cellophane and nail pieces (Arnold, 1968). The isolated species were identified using following literature: Middleton (1943), Raper & Thom (1949), Cooke (1963), Raper & Fennel (1965), Coker (1969), Seymour (1969), Gilman (1971), Barnet & Hunter (1972), Batko (1975) etc.

CHARACTERISTICS OF RESERVOIRS

Ćelije reservoir is located on the river Rasina, 30 km away from the city of Kruševac. Constructed in 1979, it has a drainage basin of 598 km2 and the reservoir itself has a surface of 60 km2, a volume of around 60×10^6 m^3 and maximum depth of 40 m. The current purpose of Ćelije reservoir is to supply only the city of Kruševac with drinking

water, however it will soon be used for the same purpose serving the neighbouring cities: Paraćin, Ćuprija and Jagodina. Based on the level of primary organic production and analysis of plankton species as indicators of trophic conditions, it can be concluded that the lake belongs to the mesotrophic type (Seleši, 1989; Ranković *et al.*, 1992).

Grošnica lake was made in 1931 by building a dam on the Grošnica river, right affluent of Lepenica river. It is located in the vicinity of Kragujevac and is used for supplying this city with water. Initial volume of the lake was 1.7×10^6 m^3. The dam height was increased by 7 m in 1962, thus increasing the lake volume to 3.1×10^6 m^3. The total dam lenght is 138 m, and its height is 50 m. Based on the indicators of water trophic conditions, it can be concluded that the lake belongs to the mesotrophic type.

Gruža reservoir was constructed in 1983 by building a dam 26 m high in the middle part of the Gruža river drainage basin. Its basic purpose is to supply drinking water for Kragujevac, Kraljevo and surrounding places. The reservoir is located at an altitude of 238-269 m. Its total volume in the period of investigation was $64{,}6 \times 10^6$ m^3 and the surface area was 934 ha. Gruža reservoir has a drainage basin of 318 km, and maximum depth of 31 m. Based on the level of primary organic production and analysis of plancton species as indicators of trophic conditions, it can be concluded that the lake belongs to the eutrophic type (Martinović-Vitanović & Kalafatic, 1990).

RESULTS AND DISCUSSION

Qualitative and quantitative composition of the fungal community

Having analized the fungal community density in the studied reservoirs during the first season, the following conclusions were reached (Tab. 1). Average number of spores in the water of different reservoirs varied significantly. Results of the statistical analysis show that the greatest average number of spores (4990 in one liter of water) was found in the eutrophic Gruža reservoir at the location (L3), and the lowest one (2000 in one liter of water) was found in the oligo-mesotrophic Ćelije reservoir at the location (L2). The greatest quantity of spores was found in the coastal areas rich with macrophytes (L3). Number of spores in all reservoirs is higher in the lower water layers (near the bottom — L1) than in the middle water layers in the central parts of the lakes (L2). Increase of the spore number at the lower water layers of the reservoirs (L1) is the result of flushing the sediments from the bottom. Very high number of spores was observed at the lower water layers of Gruža reservoir (L1), probably as a result of the fact that at the bottom of this reservoir there is a high quantity of silt rich with organic compounds, suitable for growth of some fungi.

By monitoring the seasonal dynamics of the number of spores, it can be observed that it is the highest in the water samples taken in March and October. This can lead to the assumption that the increased number of fungi in this period is related to atrophy of macrophytic vegetation as well as with inflow of soil fungi into the lakes in the period of stream torrents. Besides, in the fall and winter periods, due to greater and more frequent movements of water flows, higher number of fungi from the silty bottom of lakes can enter the free water flows. The highest number of spores was observed in March on all locations of the studied reservoirs (Tab. 2). The maximum average number of spores was found on the location (L3) of Gruža reservoir (8320 spores/l of water). The minimum

RESERVOIR	AVERAGE NUMBER OF SPORES/LITER OF WATER		
	L1	L2	L3
Celije	2700	2000	2400
Grosnica	3300	2250	4050
Gruža	4100	3700	4990

Table 1. Number of fungal spores in the water of reservoirs in Serbia

RESERVOIR	Sampling location	Average number of spores/one liter of water			
		MARCH	JUNE	AUGUST	OCTOBER
CELIJE	L_1	4832	571	2195	3202
	L_2	3218	251	2032	2499
	L_3	3724	318	2840	2718
GROSNICA	L_1	6245	714	3166	3075
	L_2	3712	418	1920	2887
	L_3	6110	518	4219	5353
GRUŽA	L_1	6917	495	3281	5707
	L_2	5375	605	3747	5073
	L_3	8320	619	4945	6076

Table 2. Seasonal dynamics of the number of spores in the water of reservoirs in Serbia

NO	FUNGAL SPECIES	ČELIJE			GROŠNICA			GRUŽA		
		L1	L2	L3	L1	L2	L3	L1	L2	L3
1.	*Absidia spinosa* Lendn.								A	A
2.	*Alternaria alternata* (Fr.) Keiss.	J		JO	M	O		M		
3.	*Aspergillus amstelodami* (Mang) Thom et Ch.							■		■
4.	*Aspergillus candidus* Link ■■ Fr.	■		■			M			
5.	*Aspergillus flavus* Link ex Fr.	JA	A	A	AO	A	JA	A	A	A
■	*Aspergillus fumigatus* Fres.			O	A			A		JA
7.	*Aspergillus niger* van Tieghem		M					■		
8.	*Aspergillus oryze* (Ahlburg) Cohn	O			M					
■	*Aspergillus* sp.						M			
10.	*Aureobasidium pullulans* (de Bary)	A			A	O		AO		O
11.	*Botrytis cinerae* Pers. ex Fr.	M			M		O	O		O
12.	*Candida albicana* (Robin) Berkhout							O		O
13.	*Chloridium chlamydosporis* Hughes							M		O
14.	*Chaetomium globosum* Kunze ex Fr.	■					M	A		
15.	*Cladosporium carpophilum* Thum.	O		O						
16.	*Cladosporium cladosporoides* (Fres) de Vries			M		J	A			A
17.	*Cladosporium herbarum* (Pers) Link ex Fr.				M	M	M	M		MJ
18.	*Cladosporium variabile* (Coke) de Vries				MO					
19.	*Cladosporium* sp.							■		M
■■	*Epicoccum purpurascens* Ehrenb. ex Schecht			AO	A	O	O	AO	A	AO
21.	*Fusarium aquaeductum* (Rab.) Sacc.							MJ	M	M
22.	*Fusarium heterosporum* Ness.	M		M			J			O
23.	*Fusarium moniliforme* Sheldon						■			
24.	*Fusarium sporotrichiella* Bilai							O	O	
25.	*Geotrichum candidum* Link	J					JA	J		J
26.	*Hansenula anomala* (Hansen) H. et P. Sydow	A	O	O			O			
27.	*Mortierella* sp.			A						
28.	*Mucor mucedo* L ex Fr.	MO	M	M	M	M	MO	M	■	MO
29.	*Mucor hiemalis* Wehmer	JA	J	M	■	MJ				
30.	*Paecilomyces varioti* Bainier				M			M		MJ
31.	*Penicillium brevi-compactum* Dierex							MJ	J	J
32.	*Penicillium citrinum* Thom	A		A						
33.	*Penicillium cyclopium* Zukal	A		A	A	A	JA	A	JA	JA
34.	*Penicillium expansum* Link									A
35.	*Penicillium funiculosum* Thom						MJ	MJ	J	JA
36.	*Penicillium thomii* Maire						A			■
37.	*Penicillium verrucosum* var. *cyclopium* (West.) Samson	AO	O	O	A		A	AO	AO	O
38.	*Phoma herbarum* West.			A	A		O			
39.	*Phoma* sp.	M			M	O		■	M	MJ
40.	*Rhizopus nigricans* Ehr.	JA						A		
41.	*Trichoderma viride* Pers. ex S. F. Gray							O		
42.	*Trichoderma* sp.				M			■		
43.	*Verticillium lateritium* Berk.				AO			A		O
44	*Verticillium terrerum* (Nees:Pers) Link				O			M		
45.	*Verticillium* sp.				M					

- Sampling period - M = March; J = June; A = August; O = October
- L1, L2, L3 are water samples taken from different water layers (see "Material and methods").

Table 3. The composition and dynamics of fungal communities in reservoirs in Serbia

NO	FUNGAL SPECIES	ĆELIJE RESERVOIR			GROŠNICA RESERVOIR			GRUŽA RESERVOIR		
		L1	L2	L3	L1	L2	L3	L1	L2	L3
1.	*Achlya americana* Humph.			AO		A	JA	MJ	MJ	AO
2.	*Achlya racemosa* Hild.					J				M
3.	*Catenaria* sp.									J
4.	*Dictyuchus sterile* Coker	MJ	JA	JA	MJ	JA	AO	AO	MJ	MJA O
5.	*Pythium ultimum* Trow	A	JA	A	MJ	J	O	O	AO	AO
6.	*Saprolegnia ferax* (Gguith) Thuret			M						M
7.	*Saprolegnia hypogyna* Pringsh.			A			AO		A	AO
8.	*Saprolegnia monica* Pringsh.						A			AO

Note:

- Sampling period - M = March; J = June; A = August; O = October
- L1, L2, L3 are water samples taken from different water layers (see "Material and methods").

Table 4. The composition and dynamics of autochthonous aquatic fungal communities in reservoirs in Serbia

average number of spores was recorded in June in all reservoirs, while the lowest one was found on the location (L2) Ćelije reservoir (251 spores/l of water). Seasonal dynamics of fungi was most prominent in the water samples taken from Gruža reservoir.

Qualitative composition of species is shown in Tab. 3. In the water samples taken from these reservoirs, 45 species were identified out of 384 isolates. It should be observed that most of them are found rarely or only once and that only representatives of some genera had a relatively high degree of mass presence. These fungi primarily belong to the genera *Aspergillus* and *Penicillium*. They are dominant in all investigations of studied reservoirs. Besides, the following genera of fungi are also frequently found: *Cladosporium*, *Fusarium*, *Phoma*, *Rhizopus*, *Mucor* and *Verticillium*. High resemblance in the fungal species composition was previously found in Skadar lake (Ristanović, 1973), where species of genus *Penicillium* were dominant, as well as in the water of Vlasina lake (Vukojević *et al.*, 1994).

Number of fungal species in the water samples taken from the studied reservoirs does not vary greatly. The greatest number of species was found in Gruža reservoir (33)

while the lowest number of species was found in Ćelije reservoir (24), which can be related to the trophic condition of reservoirs.

Fungi isolated from the water samples taken from the reservoirs were in conformance with ecological classification of aquatic heterotrophic microorganisms (Park, 1972a) and they belong to the category of transitional, accidental microorganisms and probably migrants. The latter are characterized by periodic and sporadic activity. Transitional and accidental organisms can develop sporadic activity or have a lack of it. Park (1972b) indicates the possibility of the soil fungi participation in microbiological processes in waters. Besides, living capacity of some soil fungi (species of genera *Fusarium*, *Botrytis* and *Chaetomium*) in sea and river water was experimentally proven (Alton, 1985). Thus, depending on the trophye conditions of the lake, along with the typical aquatic (autochthonous) fungi, the soil fungi can also participate in the microbiological processes present in the lake ecosystems.

Qualitative composition of the autochthonous fungal community

Out of biphlagelatic series of this group of fungi, this paper included 8 species which belong to the Saprolegniales and Peronosporales orders. The distribution results related to this group of microorganisms are given below (Tab. 4).

Most of the identified species belong to the order Saprolegniales and the dominant genera are *Achlya* and *Saprolegnia*. These species were isolated from most of the samples taken in the studied reservoirs. They were also found as dominant in the waters of American lake Blelham Tarn (Dick, 1966) and Skadar lake in Macedonia (Ristanovic, 1973). The highest number of species (8) was identified in Gruža reservoir, while the lowest number (5) was found in Ćelije reservoir, indicating that the trophic conditions of the reservoir probably affects the number of these fungi. The significant number of samples contained fungi belonging to the genera *Dictyuchus* and *Pythium*, while *Catenaria sp.* was located only in one sample taken from Gruža reservoir. By monitoring the seasonal dynamics, no regularity in fungal distribution in the studied reservoirs was found. In general, sporadic nature of the aquatic fungi distribution was prominent in some parts of the reservoirs, with their more significant presence in the coastal parts rich in macrovegetation.

Mycological studies of reservoirs are important both from the mycological and ecological aspects, since presence of certain species can be the indicator of the water quality level.

REFERENCES

ALTON L. V., 1985 — Survival of some *Fusarium* species in sea and river water. *Mycol. Phytopathol.* 19, 3: 193-199.

ARNOLD G. R., 1968 — Methods of collection and studying fresh water hyphomycetes. *Mycol. Phytophatol.* 2: 158-160.

BÄRLOCHER F., 1980 — Leaf-eating invertebrates as competitors of aquatic hyphomycetes. *Oecologia* 47: 303-306.

BÄRLOCHER F., 1981 — Fungi of the food and in the feaces of *Gammarus pulex*. *Transactions of the british mycological society* 76: 160-165.

BARNETT H. & HUNTER B., 1972 — *Illustrated Genera of Imperfect Fungi*. Burgess Publ. Co Minneapolis, 241 p.

BATKO A., 1975 — *Zarys hydromikologii*. Panstwowe Wydawnichtwo Naukowe, Warszawa, 417 p.

COKER W. C. 1969 — *The Saprolegniaceae*. The University of North Carolina Press, 201 p.

COOKE B. W., 1963 — *A laboratory guide to fungi in polluted water, sevage, and sewage treatments system*. Public Health Service. Publication 999-WP-1, 132 p.

ČOMIĆ L. J., RANKOVIĆ B. & BARBIĆ F., 1996 — Fungi in Gruža reservoir. *Archiv für hydrobiologie. Spec. Issued Advanc. Limnol.* 48: 105-111.

DICK W. M., 1966 — The Saprolegniaceae of the Environ of Blelham Tarn: Sampling Techniques and Estimation of Propagule Numbers. *Journal of general microbiology* 42: 257-282.

GILMAN I. S., 1971 — *Soil Fungi*. The Iowa State Univ. Press. USA, 450 p.

HYNES H. B. N., KAUSHIK N. R. & LOCK M. A. 1974 — Benthos and allocnthonous organic matter in stream. *Journal of the fisheries research board of Canada* 31, 5: 545-553.

MARTINOVÍC V. & KALAFATİC V., 1990 — Classification of some reservoirs in SR Serbia (SFR Yugoslavia) based on analysis of plancton species as indicators of trophic conditions. *Archiv für Hydrobiolie Beihefte., Ergebn. Limnol.* 33: 381-387.

MIDLLETON J., 1943 — *The taxonomy, host range and geographic distribution of the genus Pythium*. Memoirs of the Torrey Botanical Club, Lancaster, 171p.

PARK D., 1972a — Methods of detecting fungi in organic detritus in water. *Transactions of the british mycological society* 58, 2: 281-290.

PARK D., 1972b — On ecology of heterotrophic micro-organisms in fresh-water. *Transactions of the british mycological society* 58(2): 291-299.

RANKOVİC B., ČOMİC L. J. & SIMİC S., 1992 — *Fitoplankton akumulacionog jezera Ćelije*. Konferencija Zaštita voda 92. Zbornik radova : 90-95.

RANKOVİC B., ČOMİC L. J. & SIMİC S., 1994 — *Populacija gljiva u akumulacionom jezeru Gruža*. Konferencija Zaštita voda 94. Zbornik radova: 110 — 116.

RAPER K. B. & THOM C., 1949 — *A manual of the Penicillia*. The Williams & Wilkins Co., Baltimore, Maryland, 875 p.

RAPER K. B. & FENNEL D. I., 1965 — *The genus Aspergillus*. The Williams & Wilkins Co., Baltimore, Maryland, 875 p.

RISTANOVİC B., 1973 — The population of fungi in Skadar Lake with Special emphasis on aquatic phycomycetes. *Mikrobiologija* 10(1): 53-61.

SELEŠI D., SIMİC D. & LEHOCKI I., 1989 — *Nutritijentni elementi u akumulaciji Ćelije u blizini Kruševca*. Konferencija Zaštita voda 89. Zbornik radova: 98-107.

SEN B., 1988a — Fungal parasitism of planktonic algae in Shearwater III. Fungal parasites of diatoms. *Archiv für Hydrobiologie, Suppl.* 79(2-3): 167-175.

SEN B., 1988b — Fungal parasitism on planctonic algae in Shearwater V. Fungal parasites of the green algae. *Archiv für Hydrobiologie, Suppl.* 79(2-3): 185-205.

VUKOJEVİC J. & FRANİC-MIHAJLOVİC D., 1994 — *Mikopopulacija Vlasinskog jezera i slivnog podrucja*. Konferencija Zaštita voda 94. Zbornik radova: 210-214.

SEYOMOUR R. I., 1969 — *The genus Saprolegnia*. Beihefte zur nova hedwigia, 124 p.